The role of exosomes and microflora in establishing mucosal tolerance and the protection against allergic disease

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Göteborgs Universitet kommer att försvaras offentligt i Föreläsningssalen, våning 3, Guldhedsgatan 10A, Göteborg

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Avhandlingen baseras på följande arbeten:

I. Lin XP, Almqvist N, Telemo E.
Human small intestinal epithelial cells constitutively express the key elements for antigen processing and the production of exosomes.
Blood Cells Mol Dis 2005;35(2):122-8.

II. Almqvist N, Lönnqvist A, Hultkrantz S, Rask C, Telemo E.
Serum-derived exosomes from antigen-fed mice prevent allergic sensitization in a model of allergic asthma.
Immunology 2008;125(1):21-7.

III. Almqvist N, Gerhmann U, Magnusson M, Telemo E.
Intestinal epithelial cell derived exosomes protect against an allergic sensitization and acts via pDCs in vitro.
In manuscript.

IV. Hultkrantz S, Almqvist N, Lönnqvist A, Östman S, Rask C, Telemo E, Wold A.
S. aureus enterotoxin facilitates tolerogenic processing of mucosally administered antigens.
In manuscript.

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The role of exosomes and microflora in establishing mucosal tolerance and the protection against allergic disease

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The breakdown of immune regulation to innocuous environmental antigens at mucosal sites can result in a number of different diseases such as, allergies and inflammatory bowel disease (IBD). Allergy is one of the most common diseases with a prevalence of up to 40% in children from developed countries. The healthy immune system prevents allergic sensitization by establishing immunological tolerance to innoccus antigens present at mucosal sites.

Oral administration of soluble protein antigens is a very effective way to establish antigen-specific tolerance to the ingested protein, a process known as oral or mucosal tolerance. This is an active process, which is maintained by the specific recognition of antigens by $CD4^+$ T-cells with a down regulatory function, and it is the default response to harmless antigens entering at mucosal sites. The process of oral tolerance starts with sampling of luminal antigens by the intestinal epithelial cells (IEC), processing and assembly with MHC II and subsequently a release of tolerogenic exosomes, small (40-90 nm) membrane bound vesicles of endocytic origin, produced by intestinal epithelial cells (IEC) and can be isolated from serum shortly after an antigen feed. We have previously shown that these exosomes potently transfer antigen-specific tolerance to naive recipients. Moreover, exosome-mediated tolerance is MHC class II dependent, which in turn requires an intact immune system in the fed donor. The hygiene hypothesis states that microbial exposure is required to properly educate the immune system. A full microbial flora in the gut generally provides the required stimuli for the maturation of the intestinal immune system and the intestinal epithelial cells to enable tolerogenic processing of orally administrated antigens. It is not known which individual bacterial species or what bacterial products that delivers the necessary signals.

The focus of this thesis was to further study the role of exosomes in oral tolerance and their capacity to protect against an allergic sensitization and whether microbial stimuli would effect the outcome of such response. We also wanted to examine the role of dendritic cells in exosome-induced tolerance, focusing on plasmacytoid dendritic cells (pDC).

We found that exosomes both isolated from serum and when isolated from intestinal epithelial cells in culture protect against an allergic sensitization in an antigen-specific manner. We could also show that the tolerant animals had higher levels of activated regulatory T cells in the draining lymph nodes indicating that exosome-induced tolerance is most likley mediated by regulatory T cells. Furthermore, we could also show that the tolerogenic effect of exosomes from serum could be enhanced when the gut epithelium was exposed to enterotoxin from *S. aureus* (SEA). When investingating the uptake of IEC derived exosomes by dendritic cells we could show that both conventional dendritic cells (cDC) and pDCs phagocytose exosomes. The capacity of pDCs to phagocytose have been questioned but our results indicate that they most readily ingest both exosomes and latex beads the size of exosomes. We also compared the capacity of the DCs to process and present the antigens carried by exosomes and found that pDCs induce higher antigen-specific T cell proliferation as compared to cDCs which suggest that pDCs in fact are better at both phagocytosis of IEC derived exosomes as well as presenting the antigen they carry.

In conclusion, exosomes have the capacity to induce antigen-specific tolerance and protect against allergy. This exosome-induced tolerance could possibly be mediated by pDCs. Furthermore, in agreement with the hygiene hypothesis we could conclude that certain microbial stimuli, here SEA, does effect the tolerogenic processing, due to a more activated immune system in the gut.